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Enhanced peroxidase-like activity of porphyrin functionalized ceria nanorods for sensitive and selective colorimetric detection of glucose

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ABSTRACT

Ceria nanorods modified with 5,10,15,20-tetrakis(4-carboxyl phenyl)-porphyrin (H_2TCPP) were prepared. These nanocomposites (H_2TCPP–CeO_2) exhibited the intrinsic peroxidase-like activity and could catalyze the oxidation of classical peroxidase substrate 3,3′,5,5′-tetramethylbiphenyl dihydrochloride (TMB·2HCl) in the presence of H_2O_2 to produce a typical color reaction from colorless to blue. Our results demonstrated that both the H_2TCPP–CeO_2 nanocomposites and CeO_2 nanorods exhibited higher thermal durance than that of HRP. The affinity of The H_2TCPP–CeO_2 nanocomposites toward H_2O_2 and TMB is similar to that of HRP. Fluorescent results indicated that the catalytic mechanism of the H_2TCPP–CeO_2 nanocomposites were from the decomposition of H_2O_2 into hydroxyl radicals. Based on these studies, a simple, sensitive, and selective visual and colorimetric method using TMB as the substrate was designed to detect glucose when combined with glucose oxidase. The proposed colorimetric method can detect H_2O_2 at a low detection limit of 6.1×10^{-6} M and a dynamic range of 10^{-5}–10^{-4} mol·L^{-1}. This method can also detect glucose at a low detection limit of 3.3×10^{-5} mol·L^{-1} and a dynamic range of 5.0×10^{-5}–1.0×10^{-4} mol·L^{-1}.

1. Introduction

Enzymes are efficient biological catalysts, which are involved in almost all reactions. Natural enzymes with high substrate specificity and high catalytic efficiency play central roles in a wide range of applications, such as clinical diagnosis, biotechnology, chemistry, and environmental science, and have been extensively studied for more than 200 years [1–4]. Unfortunately, the intrinsic drawbacks of their easy denaturation [5] and high costs in preparation and purification [6] limit their large scale applications. To overcome the limitations of natural enzymes, intense interest has grown in the development of non-enzymatic glucose sensors [7a] and artificial enzyme mimetics [7b]. Nanomaterials are attractive and are widely put to use because of their unique optical, electronic, magnetic, and catalytic properties [8]. In 2007, the excitingly intrinsic peroxidase-like activity of ferromagnetic nanoparticles, an inert nanomaterial, was reported [9] and aroused people's attention for the innovative research of nanomaterials in the enzyme mimetic field [10]. Various nanoparticles have been evaluated as enzymatic mimetics [11–39], including ferromagnetic NPs with peroxidase-like activity [11–20], ceria oxide NPs [21–24] and V_2O_5 nanowires [25] with oxidase mimetic properties, metal nanoparticles with oxidase or peroxidase-like activity [26–31], SiO_2/PEG hybrid materials [22] and carbon-based nanomaterials. [23–39].

Nanoceria, as an important rare-earth oxide nanostructure material, has attracted enormous interest in recent years, due to the unique physical and chemical properties compared with that of its bulk materials. Therefore, it has been widely applied in various areas, including catalysis, electrochemistry, photochemistry, metal polishing agent, fuel cells, gas sensors and luminescence [40–46]. In recent studies, cerium oxide nanoparticles (nanoceria) are found to be the potent free-radical scavengers as well as exhibit neuroprotective, radioprotective, and anti-inflammatory properties [47–49]. Nanoceria also have the unique property of being regenerative or autocatalytic [50]. In addition, the novel properties of nanomaterials are associated with their size, shape and morphology, such as sphere, rod, polyhedral, and cubic. [51–53].

Nanoceria with the higher level of Ce^{4+} oxidation state exhibited significant catalase-mimetic activity, while those with the higher level of Ce^{3+} oxidation state did not, so the decomposition of H_2O_2 to oxygen by nanoceria depended on the ratio of Ce^{3+}/Ce^{4+}, which could be modulated by morphology, H_2O_2 and phosphate solutions (e.g., phosphate buffered saline) [54] to improve catalase-mimetic activity. A new horseradish peroxidase (HRP) third-generation electrochemical biosensor based on ceria nanocubes has been established [55]. CeO_2 nanoparticles can be used to detect glucose in human blood serum samples by colorimetric method [56]. However, the ceria nanostructures that have been investigated so far for their peroxidase mimetic activity have had zero dimensional shape.

Porphyrin, tetrapyrrole derivatives as the representatives of functional molecular materials with large conjugated electronic molecular

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structures, have attracted great research interest in vastly diverse areas ranging from chemistry, physics, biology, and medicine to molecular device [57]. Porphyrins widely exist in organism and the energy transfer of the relevant important organelles. If we can obtain the functional porphyrin-ceria nanocomposites, it will provide excellent opportunities for applications in the fields of artificial enzyme mimetics, biosensor, electrocatalysis, luminescence, electronics, etc. However, to the best of our knowledge, one dimensional ceria nanorods as well as 5,10,15,20-tetrakis(4-carboxyl pheyl)-porphyrin (H_2TCPP) modified ceria nanorods have not been reported thus far.

In this paper, H_2TCPP modified CeO_2 nanorods were prepared by a facile two-step method. Interestingly, the as-prepared CeO_2 nanorods and H_2TCPP–CeO_2 nanocomposites demonstrated peroxidase-like activity that they could catalyze oxidation of the substrate 3,3′,5,5′-ertramethylbenzidine dihydrochloride (TMB·2HCl) in the presence of H_2O_2. Furthermore, the catalytic activity of H_2TCPP–CeO_2 nanocomposite showed much higher than that of pure CeO_2 nanorods. As a result, H_2TCPP–CeO_2 nanocomposites were used as mimic enzyme by a colorimetric method for H_2O_2 detection. And we all know that the oxidation of glucose could be catalyzed by glucose oxidase (GOx) in the presence of oxygen to produce gluconic acid and H_2O_2. Therefore, a simple, sensitive and cheap colorimetric method has been developed for glucose detection (Scheme 1). The results indicate that this method is higher sensitive and selective for the detection of glucose and can be successfully used in the quantitative detection of glucose in buffer solution and even in human serum system.

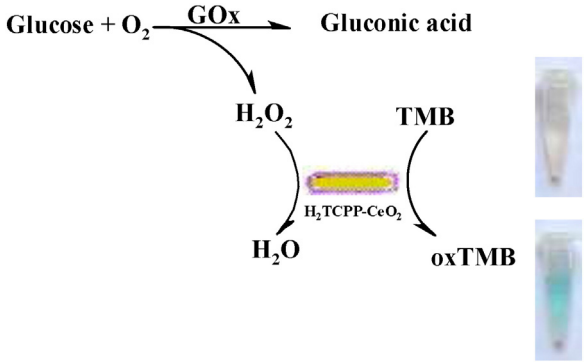
2. Experimental section

2.1. Chemicals and reagents

Cerium nitrate hexahydrate ($Ce(NO_3)_3·6H_2O$), hydrogen peroxide (30 wt.%,H_2O_2), acetic acid (HAc), sodium acetate(NaAc), TMB·2HCl, glucose, fructose, lactose, and mannitol were purchased from Guangcheng Reagent Co. (Tianjin, China). Glucose oxidase (GOx, ≥200 U mg^{-1}) was purchased from Sigma-Aldrich and stored in a refrigerator at −18 °C. All the regents were of analytical reagent grade and used without further purification. 5,10,15,20-Tetrakis(4-carboxyl pheyl)-porphyrin (H_2TCPP) was synthesized according to the previous literature [58].

2.2. Preparation of CeO_2 nanorods and porphyrin functionalized CeO_2 nanocomposites (H_2TCPP–CeO_2)

CeO_2 nanorods were prepared according to a previous report [59]. In a typical procedure, $Ce(NO_3)_3$ (8 ml, 0.05 M) was added rapidly to H_2O_2 (8 ml, 30.0%) solution and stirred for ca. 10 min. The suspension was transferred into a 25 ml Teflon-lined autoclave, which was heated at 250 °C for 3 h. The system was then allowed to cool to room temperature naturally. The final product was washed with deionized water, and dried at 60 °C for 12 h. Then CeO_2 nanorods were obtained. Subsequently, the CeO_2 nanorods was dissolved into 5 mM H_2TCPP solution (pH = 8) was treated to an ultrasonic water bath for 1.5 h. The sample (H_2TCPP–CeO_2) was washed with NaOH solution (pH = 8) and dried at room temperature in air. The target product was successfully obtained and studied in the subsequent experiment.



Scheme 1. Schematic illustration of oxidation of glucose by GOx.

2.3. Characterization

The morphology and size distribution of the nanorods were imaged by transmission electron microscope (TEM JEM-2100, JEOL, Japan). The crystal structures of the products were determined by powder X-ray diffraction (XRD) patterns with graphite monochromatized Cu Kα radiation (D/Max2500PC, Rigaku, Japan). Fourier transform infrared (FTIR) spectra were taken in KBr pressed pellets on a NICOLET 380 FT-IR spectrometer (Nicolet Thermo, USA). UV–vis absorption spectra were recorded on a MAPADA UV-3200PC spectrophotometer (Shanghai, China). X-ray photoelectron spectra (XPS) were recorded on a PHI Quantera SXM spectrometer with an Al Kα = 280.00 eV excitation source, and binding energies were calibrated by referencing the C1s peak (284.5 eV) to reduce the sample charge effect.

2.4. Mechanism of peroxidase-like activity of H_2TCPP–CeO_2 nanorods

We presume that the nature of peroxidase-like activities of the H_2TCPP–CeO_2 nanorods may originate from their catalytic ability to H_2O_2 decomposition into •OH radicals. So we used a method of fluorescence to measure the •OH radicals with terephthalic acid as probe. The typical experimental procedure is as follows: H_2O_2 (10 mM), terephthalic acid (0.5 mM) and the CeO_2 nanorods with different concentrations (0, 50, 100, 150, 200, 250, 300 μg/ml) were first incubated in acetate buffer (pH 3.8, 100 mM) at 40 °C for 40 min. After centrifugation, the solutions were used for fluorometric measurement by a Cary Eclipse spectrofluorophotometer (Varian, Inc., USA).

2.5. Kinetic analysis

The reaction kinetic measurements were carried out in time course mode by monitoring the absorbance variation at 652 nm with a 1 s interval on a MAPADA UV-3200PC spectrophotometer. The experiments were carried out with 0.04 mg·mL^{-1} H_2TCPP–CeO_2 solution or 0.04 mg·mL^{-1} CeO_2 in reaction buffer (0.026 M NaAc, pH 3.8) with TMB·2HCl as substrate; H_2O_2 concentration was 25 mM for CeO_2 and H_2TCPP–CeO_2 nanorods. The Michaelis–Menten constant was calculated according to the Michaelis–Menten eqn (1): $v = V_{max}[S] / (K_m + [S])$, where v is the initial velocity, [S] is the concentration of substrate, K_m is the Michaelis constant and V_{max} is the maximal reaction velocity.

2.6. H_2O_2 and glucose detection

In a typical process, the solution of hydrogen peroxide with a given concentration was added to TMB (final concentration of 0.25 mM) solution in the presence of H_2TCPP–CeO_2 nanocomposites solution (final concentration of 0.02 mg·mL^{-1}) and the NaAc buffer (pH 3.8). The mixture was incubated at room temperature for 10 min.

Glucose detection was realized as follows: a) 100 μl of 0.5 mg·mL^{-1} GOx and 500 μL of glucose of different concentrations in PBS (pH 7.0) were incubated at 37 °C for 0.5 h; b) 200 μL of 0.5 mM TMB, 30 μL of the H_2TCPP–CeO_2 nanocomposites solution, and 1.17 mL of buffer (pH 3.8) were added into the above 200 μL glucose reaction solution. The mixed solution was used to perform the time course measurement.